Endometrial infusion of human chorionic gonadotropin at the time of blastocyst embryo transfer does not impact clinical outcomes: a randomized, double-blind, placebo-controlled trial

Kathleen H. Hong, M.D.,[a,b] Eric J. Forman, M.D.,[a,b] Marie D. Werner, M.D.,[a] Kathleen M. Upham, B.S.,[b] Christina L. Gumeny, B.S.,[b] Ayesha D. Winslow, M.S.,[b] Thomas J. Kim, M.D.,[a,b] and Richard T. Scott Jr., M.D., H.C.L.D.[a,b]

[a] Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Reproductive Sciences, Rutgers-Robert Wood Johnson Medical School; and [b] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey

Objective: To determine whether endometrial hCG infusion at the time of human blastocyst transfer impacts implantation rates.
Design: Randomized double-blinded placebo-controlled trial.
Setting: Academic.
Patient(s): Infertile couples with the female partner less than 43 years old (n = 300) undergoing fresh or frozen ET of one or two blastocysts.
Intervention(s): Patients undergoing ET were randomized into either a treatment or a control group. The treatment group received an infusion of 500 IU of hCG diluted in ET media. The control group received a sham infusion of ET media. Infusions were done using a separate catheter less than 3 minutes before actual ET.
Main Outcome Measure(s): Sustained implantation rate: ongoing viable gestation (primary outcome) and ongoing pregnancy rate (secondary outcome).
Result(s): A total of 473 blastocysts were transferred into 300 patients. There were no differences between the two groups in sustained implantation rate (48.1% in the hCG group, 44.2% in the control group) or ongoing pregnancy rate (58.8% in the hCG group, 52.0% in the control group).
Conclusion(s): Endometrial infusion of hCG at the time of blastocyst ET does not improve sustained implantation rates.
Clinical Trial Registration Number: NCT01643993. (Fertil Steril® 2014;102:1591–5. ©2014 by American Society for Reproductive Medicine.)
Key Words: hCG, intrauterine hCG, implantation rate, pregnancy rate, embryo transfer

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Reprint requests: Dr. Kathleen H. Hong, M.D., Reproductive Medicine Associates of New Jersey, 140 Allen Road, Basking Ridge, New Jersey 07920 (E-mail: khong@rmanj.com).

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While clinical implantation and delivery rates continue to rise in couples attempting to conceive using assisted reproductive technology (ART), the process remains relatively inefficient. It remains common for euploid embryos with optimal morphokinetic parameters to be transferred into

sonographically normal endometrial cavities and still fail to achieve implantation.

While some of these failures may reflect the suboptimal endocrine milieu accompanying controlled ovarian hyperstimulation to attain multifollicular development; implantation rates are also far from perfect in recipients of oocyte donation or when transferring cryopreserved embryos where endometrial preparation and timing should be closer to physiologic.

While appropriate endocrine dynamics may assure adequate endometrial preparation and timing, the process of implantation is a paracrine/juxtacrine-mediated phenomenon that is controlled locally. Among the factors important in implantation is hCG (1). Intrauterine infusion of hCG and exposure of cultured human endometrial epithelial cells has been shown to upregulate proteins known to be involved with implantation (2, 3). This has led some investigators to suggest that endometrial augmentation with infusion of hCG might lead to enhanced implantation rates.

Mansour et al. found that intraendometrial infusion of 500 IU of hCG during cleavage-stage ET significantly enhanced implantation rates (29.5% in controls vs. 41.6% after hCG infusion) (4). While these results were most provocative, several questions remain. The hCG infusion into the endometrial cavity was dyssynchronous with the physiological timing of embryonic hCG secretion, which typically begins at the morula stage (5). At that time, the embryo would be localized to the fallopian tube in natural conception, making it an unusual time to provide a paracrine signal.

Blastocyst ET (day 5 or 6 of embryo development) is becoming more prevalent, in particular to enhance selection for elective single ET (eSET). Furthermore, with the broad clinical application of vitrification, the practice of frozen ET (FET) has become increasingly common.

To date there are no published studies on the impact of endometrial hCG infusion in the perinidatory interval to determine whether the benefit identified at the cleavage stage extends to transfers done at the blastocyst stage. Prior studies also have not evaluated the impact of hCG infusion in FET cycles, which may be different from its impact in fresh ETs. This randomized controlled trial seeks to determine whether hCG infusion in the minutes before blastocyst transfer meaningfully impacts implantation and delivery rates in fresh and FET cycles.

MATERIALS AND METHODS

Patient Population

All patients undergoing fresh or frozen ET within the ART program where the female partner was less than 43 years of age were offered participation. Patients were recruited by the clinical research team and recovery room staff. Patients could not be simultaneously participating in another prospective clinical trial at the center, but there were no other inclusion/exclusion criteria. Specifically, there were no restrictions based on any aspect of clinical care before or after the infusion and transfer. All embryos are cultured until day 6 regardless of patient age or the size or quality of the embryo cohort. All fresh transfers within the program occur at the blastocyst stage on day 6 of embryonic development. In FET cycles, once an adequate endometrial thickness and pattern have been obtained, typically at least 7 mm and trilaminar, IM P in oil is started and FET is performed on the sixth day of P administration. Patients, in consultation with their physicians, elect between transfer of one or two blastocysts. Per practice routine, real-time quantitative polymerase chain reaction–based comprehensive chromosome screening (CCS) was offered to all patients (6, 7). Patients of advanced reproductive age or with a history of failed implantation were encouraged to incorporate CCS before ET. Patient enrollment extended from August 2012 to December 2013. All participants were followed clinically until their final disposition: pregnancy test for those who failed to conceive, 8 gestational weeks if pregnant with normal growth, or through the time of any pregnancy loss. Patients with ongoing gestations were discharged to their obstetricians for ongoing care, and final outcomes were then assessed after delivery. All data collection was performed at Reproductive Medicine Associates of New Jersey.

Experimental Design

A random number function was used to create variable blocks of four to eight with patients assigned to the two groups in a 1:1 allocation. Allocation concealment was achieved using sequentially numbered, opaque, sealed envelopes. Two sets of randomization schemes were used: one for fresh ET and one for FET. The study group received endometrial infusion of ET media (synthetic serum substitute and Medicult BlastAssist from Origio) laden with 500 IU of purified-urinary placental hCG (Novarel, Ferring Pharmaceuticals), and the sham control group received endometrial infusion of ET media only.

An embryologist opened the randomization envelope on the afternoon before the day of planned ET to allow time for preparation and equilibration for the next day's use. The embryologist prepared the infusion mixture by dissolving 20,000 IU of urinary hCG powder with 0.8 mL of ET media. The mixture was then stored in a preequilibrated tri-gas incubator. While there are no definitive studies demonstrating that there is equivalent potency of hCG at 37°C (temperature of culture incubator), times in vivo do not produce meaningful degradation of the molecule, nor are there any data to suggest that such a diminution would have occurred.

The usual steps were taken to prepare for ET. The patient was positioned, and a speculum was placed to visualize the cervix. The embryologist loaded 20 μL of the ET media with or without hCG into a Wallace catheter and handed it to the physician, who then advanced it into the cavity under direct ultrasound visualization to the approximate depth of the actual ET that would follow. The media were infused into the endometrial cavity, and the catheter was discarded. The embryologist then used a new Wallace catheter to load the embryo(s) in 20 μL of ET media and handed it to the physician who then performed the transfer per standard protocol. The speculum was removed immediately afterwards. The time between the infusion and ET was less than 3 minutes. Both the physician performing the transfer and the patient were

blinded to the assigned treatment group throughout the entirety of the study.

Statistical Analysis

The primary study outcome was the sustained implantation rate per embryo. A sustained implantation was defined as a transferred embryo that reached a viable gestational age (≥24 weeks). Secondary outcomes included the sustained implantation rate per embryo per age group, ongoing pregnancy rate per transfer, and clinical loss rate per transfer, as defined by sonographic evidence of a pregnancy that did not result in a sustained implantation. All rates were compared by a χ^2 distribution and by calculating 95% confidence intervals (95% CI) of the relative risk (RR) and risk difference. An alpha error of less 0.05 was considered significant.

A sample-size calculator tool (Open Epi; www.openepi.com) determined that 778 embryos would be required to detect an absolute improvement of 10% in sustained implantation rate from the baseline of 50% with a power of 80% and an alpha error of 0.05.

The protocol was approved by the Institutional Review Board and registered with clinicaltrials.gov (NCT01643993) before patient enrollment.

RESULTS

A total of 325 patients elected to participate in the study and were randomized. Twenty-five patients declined to participate after randomization for various reasons; therefore 300 patients received the planned intervention (Supplemental Fig. 1). A total of 473 embryos were transferred for a mean transfer order of 1.6 embryos. Comprehensive chromosome screening was used by 42.3% of study participants.

The mean age of the population was 35.1 years (range, 23.9–42.8 years). Of these, 51% (153/300) were less than 35 years old, 20% (60/300) were 35–37 years old, 21.7% (65/300) were 38–40 years old, and 7.3% (22/300) were 41–42 years old.

A total of 148 patients were randomized to receive endometrial infusion of the hCG-laden ET media (hCG group), and 152 patients were randomized to receive the sham infusion of ET media only (control group). Between the two groups, there were no differences in age, mean number of embryos transferred, or the percent using CCS (Table 1).

TABLE 1

The study population was similar in the two groups.

Variable	Total	hCG	Sham
Patients (n)	300	148	152
Age (y), (mean ± SEM)	35.1 ± 0.2	35.0 ± 0.3	35.1 ± 0.3
Embryos transferred (n)	473	233	240
% Using aneuploidy screening	42.3	44.6	40.1
% Fresh ET	43.2	44.7	44.0
% SET	42.6	42.1	42.3

Hong. hCG endometrial infusion at transfer. Fertil Steril 2014.

Clinical Outcomes

The primary endpoint of the study was sustained implantation rate per embryo transferred. There were no differences in sustained implantation between patients in the group receiving hCG infusion (112/233; 48.1%) and those in the control group (106/240; 44.2%; P=.39, RR = 1.13; 95% CI, 0.93–1.38; Fig. 1).

Although the study was not powered for subgroup analysis within age groups, sustained implantation rates within each age group in the study and control groups were equivalent for age ≤35, P=.73, RR = 1.04, 95% CI, 0.83–1.31; age 35–37, P=.72, RR = 1.11, 95% CI, 0.63–1.98; age 38–40, P=.61, RR = 1.12, 95% CI, 0.73–1.72; and age 41–42, P=.66, RR = 1.65, 95% CI, 0.50–5.42 (Supplemental Fig. 2).

A secondary endpoint was the overall ongoing pregnancy rate per transfer. Here again, intraendometrial infusion of hCG had no impact on clinical outcomes. The ongoing pregnancy rate per transfer was 58.8% (87/148) in the hCG group and 52.0% (79/152) in the control group (P=.24, RR = 1.09, 95% CI, 0.90–1.32), which were equivalent (Fig. 2).

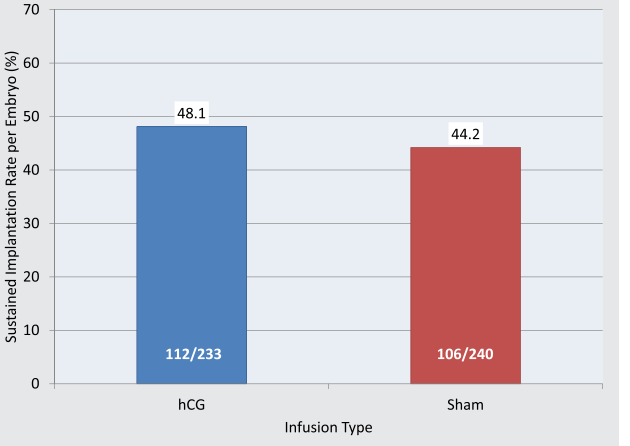
Clinical loss rates were also similar in both groups, demonstrating that infusion of hCG or ET media did not influence clinical losses. The loss rate per transfer was 11.5% (17/148) in the hCG group and 7.2% (11/152) in the control group (P=.21, RR = 1.59, 95% CI, 0.77–3.27).

Fresh IVF versus Cryopreserved ETs

The data were then stratified into those undergoing fresh ET and those involving the transfer of cryopreserved embryos with exogenous E_2 and P support of the endometrium.

A total of 227 embryos were transferred to 132 patients in fresh IVF cycles. There were no significant differences in the sustained implantation rates in the hCG infusion and control groups. Specifically, 52.7% (59/112) of embryos in the hCG group and 48.7% (56/115) of the embryos

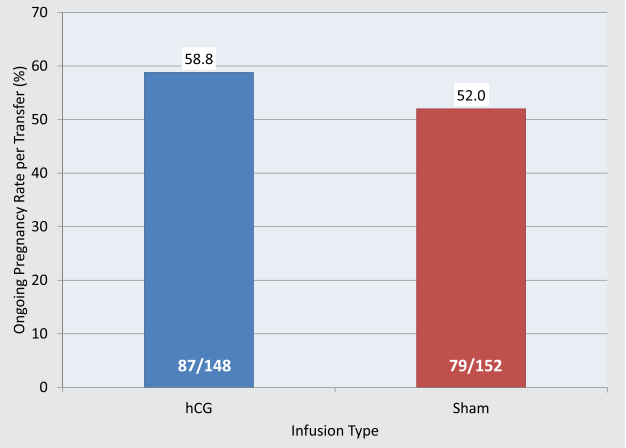
FIGURE 1



Sustained implantation rate per embryo was similar in the hCG group when compared with the sham control group.

Hong. hCG endometrial infusion at transfer. Fertil Steril 2014.

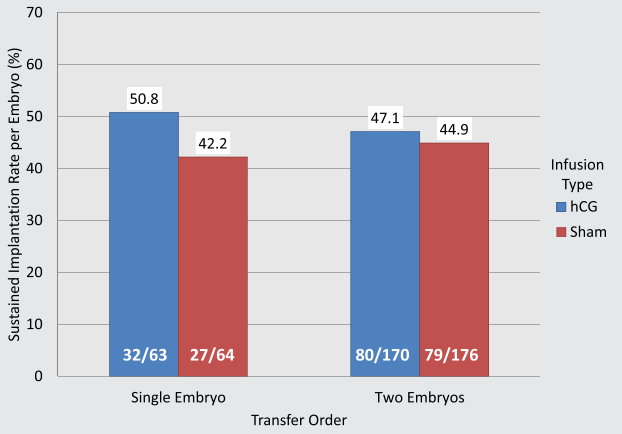
FIGURE 2



Ongoing pregnancy rate per transfer was similar in the hCG group when compared with the sham control group.

Hong. hCG endometrial infusion at transfer. Fertil Steril 2014.

FIGURE 3



Sustained implantation rates per embryo were similar in groups of similar transfer order.

Hong. hCG endometrial infusion at transfer. Fertil Steril 2014.

transferred in the control group implanted and progressed to a viable gestational age (P=.55, RR = 1.09, 95% CI, 0.84–1.40). Ongoing pregnancy rates were also similar in the hCG group (68.8%, 44/64) and in the control group (58.9%, 40/68; P=.24, RR = 1.17, 95% CI, 0.90–1.51) in fresh ET cycles.

Evaluation of FET cycles also demonstrated a lack of benefit to intraendometrial hCG infusion. Of the 246 embryos transferred into 168 patients in FET cycles, sustained implantation rates were 43.8% (53/121) in the hCG group and 40.0% (50/125) in the control group (P=.55, RR = 1.10, 95% CI, 0.82–1.47). Ongoing pregnancy rates were also similar in the hCG group (51.2%, 43/84) and in the control group (46.4%, 39/84; P=.54, RR = 1.10, 95% CI, 0.81–1.50).

Transfer Order

The number of embryos to be included in each transfer was at the discretion of the patient and the clinical team and therefore was not controlled for within the study. Thus, it was possible for the groups to have a different prevalence of SET and double ET. While not impacting the calculation of sustained implantation rate per embryo transferred, differences might impact the overall pregnancy rate per transfer since transfer of two equivalently selected embryos consistently produces a higher pregnancy and delivery rate.

Among the patients randomized to hCG infusion, 16/48 (33%) had SET, whereas 21/47 (45%) of those randomized to the control group had a SET.

Of the 127 embryos used in SETs, sustained implantation rates were 50.8% (32/63) in the hCG group and 42.2% (27/64) in the sham control group (P=.34, RR = 1.20, 95% CI, 0.83–1.75). The remaining 173 patients had two embryos replaced at ET. Sustained implantation rates were 47.1% (80/170) in the hCG group and 44.9% (79/176) in the sham control group (P=.69, RR = 1.05, 95% CI, 0.83–1.32; Fig. 3).

Euploid ETs

A total of 127 patients underwent transfer of 166 chromosomally screened embryos. There were no differences in outcome between the two groups; implantation rates were 50.6% (43/85) in the hCG group and 48.1% (39/81) in the sham control group (P=.38, RR = 1.05, 95% CI, 0.77–1.43).

Study Completion

After 300 ETs had been completed after randomization, comprising 473 transferred embryos, a planned interim safety analysis was performed to assure that there was no adverse effect from the hCG infusion. At that point in the study, 58% power to detect the goal of an absolute difference of 10% in implantation rates had been attained. A futility analysis was performed, demonstrating that in order for hCG infusion to achieve a 10% benefit in implantation rates over the sham infusion, the implantation rates in the hCG group would need to achieve a greater than 20% increase (which is a 40% relative increase) in implantation rates for the remainder of the study. Since it is exceedingly unlikely for any one parameter or confluence of variables to cause such a dramatic change, the decision was made that continuation of the study was futile and enrollment was closed.

DISCUSSION

With improvements in the efficacy and safety of ART, there has been a continued effort to identify interventions that optimize IVF outcomes with the goal of performing effective eSET across age groups. The application of extended culture has been shown to enhance selection and improve implantation rates, in particular when eSET is performed (8, 9). Trophectoderm biopsy with CCS has further enhanced eSET by allowing selective transfer of only euploid blastocysts (10). Despite these interventions, some euploid embryos still fail to implant. Potential causes limiting implantation may

be related to endometrial factors, in particular the complex embryo/endometrial interactions that occur at the time of implantation.

Ultimately, this randomized control study demonstrates that infusion of 500 IU of hCG at the time of blastocyst transfer does not significantly improve sustained implantation or delivery rates. Based on experimental in vitro data, the blastocysts undergoing ET in this trial would already be expected to be secreting hCG. It is possible that the additional hCG supplementation at this stage was insufficient to cause an improvement. It is conceivable that an enhanced signal to the endometrium may not be sufficient to enhance the performance of embryos with limited reproductive potential. Additional studies are needed to identify why embryos that otherwise appear optimal fail to implant.

While investigators continue to seek ways to improve embryo selection and optimize the endometrial milieu, additional signals from the embryo or endometrium may be identified. However, increasing the quantity of a missing or diminished cofactor does not necessarily result in improved outcomes. Additionally, interventions demonstrated to be beneficial at the cleavage stage should be cautiously assessed before implementation at the blastocyst stage. It is possible that endometrial infusion of hCG may be beneficial before cleavage-stage ET; however, it is unlikely that this approach could be used to perform effective eSET across age groups and improve the safety of IVF. The current trial cannot assess whether infusion of hCG at an earlier stage, such as on day 3, would enhance implantation of embryos transferred at the blastocyst stage. However, this would require an additional intervention and was felt to not be a practical model in this study design.

There are other differences, besides the stage of ET, in the current trial and in the one reported by Mansour et al. that may also contribute to the difference in conclusions. For one, the patient populations were different: Mansour et al. (4) included young women with male factor infertility undergoing their first IVF cycles, whereas the current study included older patients undergoing ETs without regard to the number of prior failed IVF cycles. However, any potential benefit of hCG infusion would be expected to be even more pronounced in a population at higher risk for implantation failure.

There were also slight differences in the ET technique. Mansour's group performed cervical compression for several minutes after the initial hCG infusion was performed; however, there was no visible evidence of fluid refluxing from the ectocervical os in the current trial and cervical compression is not routinely performed in this program. Since the mock transfer was performed under ultrasound guidance, it is highly unlikely that the hCG infusion did not reach the desired location.

While this study was terminated after 300 ETs and it was determined that hCG would not improve the outcome of blastocyst ETs, the possibility exists that a focused study of hCG infusion could meaningfully improve the outcome of eSET. While sustained implantation rates were not significantly different in the subset of patients undergoing eSET, this study was not powered to address this specific subgroup independently. As patients undergoing eSET comprised a larger proportion of the patients randomized to the control group in fresh ETs, this also caused ongoing pregnancy rates to appear higher in the fresh ET group, an effect that does not persist upon controlling for transfer order. Nonetheless, it may be worthwhile to explore the impact of hCG infusion in eSET in future studies.

Like any clinical trial, the current findings apply to the population studied and may not be applicable in different clinical settings or patient subgroups. Additionally, the small differences in outcomes between the study and control groups found in this trial might be significant if the differences persisted with a much larger sample size; all data are limited to the power provided by the number of test subjects included. Nevertheless, this data set provides strong evidence that routine inclusion of hCG infusion before blastocyst-stage ET, whether fresh or frozen, is not beneficial. This trial also does not address the potential impact of the infusion itself, as it was designed to isolate the impact of hCG, which necessitates performance of a sham infusion in the control group. Further study would be required to determine whether infusion alone alters outcomes positively or negatively.

While there is clear evidence that hCG plays an important role in the perinidatory interval in both animals and humans, these data do not support its augmentation at the time of embryo transfer.

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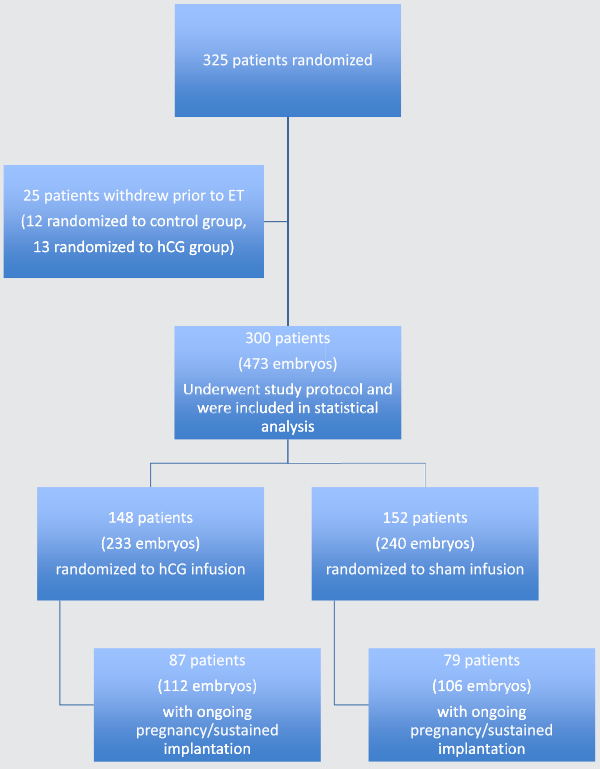
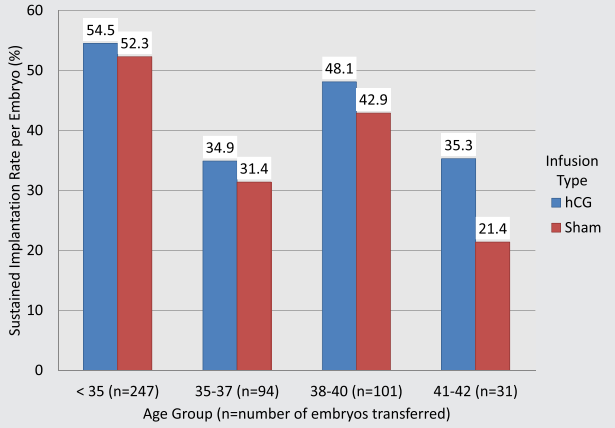
SUPPLEMENTAL FIGURE 1



Study flow. Of the 325 patients who were randomized, 300 underwent the treatment protocol, and 100% of the patients who underwent the study protocol were included in the data analysis. There was no loss to follow-up.

Hong. hCG endometrial infusion at transfer. Fertil Steril 2014.

SUPPLEMENTAL FIGURE 2



Sustained implantation rates per embryo per age group. There were no statistically significant differences between the study groups when stratified by age group.

Hong. hCG endometrial infusion at transfer. Fertil Steril 2014.